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COMPARATIVE EVALUATION OF ALTERNATIVE MOLECULAR ARCHITECTURES FOR ERCAS12A-BASED MULTIVALENT CRISPR-ACTIVATOR SYSTEM IN CHO CELLS

Background. Catalytically inactive Cas12a (dCas12a) enables robust design of multiplexed CRISPR activators (CRISPRa) through its innate crRNA-array processing. Coupling Cas12a-CRISPRa with multivalent activation scaffolds concentrates transcription activator domains (TADs) at target promoters, driving efficient, position-invariant gene upregulation. dErCas12a-based CRISPRa are promising IP-friendly effectors for commercial cell line engineering, yet no dErCas12a-based multivalent activator designs have been characterized. **Aim.** Compare activity of multivalent scaffolds based on GCN/ALFAtag affinity tags at varying multivalency and alternative TADs for dErCas12a activator in a cell-based transcriptional reporter assay. **Methods.** All fusion proteins were expressed as mCMV-driven constructs. dErCas12a linked to 10x/3xALFAtag or 10x/3xGCN repeats was combined with TADs (VPR or P65-HSF1) fusion to ALFAtag or scFv nanobody respectively. Direct dErCas12a-VPR fusion served as positive control. A pre-validated crRNA targeting miniCMV was U6-expressed from a separate vector. CHO-K1 cells expressing miniCMV-driven CyOFP1 were electroporated in triplicate with three constructs: tag-fused nuclease; matched TAD; crRNA cassette. At 72h activation was analyzed by flow cytometry. Box-Cox-transformed ($\lambda = -0.203$) activity, normalized to nuclease and activator reporters signals, was modelled with a two-level full-factorial model including all three factors and two-factor interactions. **Results.** The highest activity was observed for ALFAtag-based activators: 12.5-fold for 3X-VPR and 9.5-fold for 10X-P65-HSF1; versus 6.5-fold for the dErCas12a-VPR control. The reduced factorial model ($R^2_{adj} = 0.978$, $pLOF = 0.14$; standardized effect size (SES) mentioned for each factor below) retained all factors as significant, identifying ALFAtag (20.4982 SES) and VPR (18.0275 SES) as the strongest contributors to high specific activity designs. The much stronger interaction terms for multivalency with both the affinity tag (10.7128 SES) and the TAD (10.3193 SES), compared to the single term effect (4.1179 SES), show that TAD multimerization depends strongly on the construct architecture. All conditions showed significant viability reduction correlating with specific activity, suggesting a complex toxicity profile. **Conclusions.** For the first time, multivalent-scaffold dErCas12a-CRISPRa systems were demonstrated, outperforming classical direct-fusion systems. Future designs will require careful optimization of multivalency for specific TADs to maximize specific activity with reduced transcriptional toxicity.

Keywords: CRISPRa, ErCas12a, VPR, P65-HSF1, multivalent activator.

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